EMC Project # 23

Fixation of Chicken Egg Blastoderm and Embryo

Fixation Procedure:

After opening eggs, the albumin was removed from the outer vitline membrane. Small pieces of 20 micron nylon mesh were placed over selected areas, and then a piece of round mesh 1.5mm nylon was laid over the whole area. A polyethylene vial cap with top cut out was placed over the mesh with edge making contact with the vitline membrane. The cap would tightly adhere and thus make a sealed reservoir to hold the fixative solution. The fixative solution was pur into the reservoir and the egg placed into a refrigerator. After fixing for 1/2 - 1 hour, the specimen and cap were cut l loose from the egg. Excess yoke was carefully removed by wiping gently with a K-wipe. The cap was then put back into fresh fixative in a small plastic petri dish and put back into the refigerator. The tissue was left for another 4 hour . All the fixative was removed and replaced with 0.1M buffer in the cold for 30min. Fresh buffer replaced with the buffer rinse and was left for overnite to 24hr. The buffer was replaced with 2-3% osmium tetroxide in H_2O and left at 10C for 2 hr. The osmium was washed-- removed the tissue rinsed with H_2O for 10min. followed by alcohol dehydration.

EMC - 23 A Fresh eggs incubated 24 hr.
EMC - 23 B Fresh eggs stored 24 hr.
EMC - 23 C 2 Week stored eggs incubated 24 hr.
EMC - 23 D 2 week stored eggs
EMC - 23 E 4 week stored eggs incubated 24 hr.
EMC - 23 F 4 week stored eggs

Fixative Solution:

8% glut. in 0.1M s-collidine buffer.10min. @ rm. temp. then 1-4 hr. @ 10C

Buffer Solution:

0.1M s-collidine buffer @ 10C for overnite to 24hr.